

Comparative study of survival and growth performance of *penaeus vannamei* (Boone, 1931) with probiotic supplementation at higher salinities

UNDER PEER REVIEW

ABSTRACT

Probiotics are considered as one of the most significant components of shrimp aquaculture due to its several beneficial effects such as disease prevention, water quality maintenance, and growth performance. Present research work was designed to compare the performance of *Penaeus vannamei* at different hypersaline condition under probiotics supplementation. A 60 days experiment trial was carried out at higher salinities (45, 50, 55 and 60 ppt) with probiotic. All the important water quality parameters such as total ammonia nitrogen, nitrite, total hardness, total alkalinity, pH etc. was found to be in optimum range after few weeks in all treatments and control but the effect was better in control (45 ppt) and T-1 (50 ppt). The growth performance of *P. vannamei* was found better in control and T-1 compared to the rest of the treatments. FCR was found to be significantly lower in control compared to T-3. A similar trend was observed with survival percentage. Average body weight gain, protein efficiency ratio, feed efficiency ratio and specific growth ratio was found better in control and lowest in T-3 (60 ppt). Hence it can be concluded that 45 and 50 ppt with probiotics is most suitable for culture as compared to other higher salinities.

Keywords: - *P. vannamei*, Higher salinity, probiotics, growth performance & survival

1. INTRODUCTION

Aquaculture is considered one of the fastest growing foods producing sector and presently contributing around 47% of world total food fish production (FAO, 2018). Indian aquaculture is undergoing rapid developments towards achieving the goal of blue revolution and holding the second rank in the world aquaculture, with the production of 5,066 thousand tonnes in 2016 (FAO, 2018). The current pace of aquaculture is evident due to extensive transformations happening within the industry, mainly due to the adoption of an advanced strategy of water quality, feed and disease management. It further opens the scope of vertical expansion and shows the hope to provide nutritional security to rapidly growing populations all around the world. Shrimp farming is the key player which has changed the face of aquaculture around the globe. Indian shrimp aquaculture industry has undergone remarkable transformations during the last 10 years and same is reflected in export performance which is 65% of total seafood export in the country during 2016-17 (Ayan, 2017). The total export shrimp contributes approximately. 34,484 metric tons (US\$ 3726.36 million) in 2016-17 more than half of the total marine product export of the country. Out of the total shrimp export from the country during 2016-17 *Litopenaeus vannamei* contributes about 28.46 % (quantity) and approx. 50% contribute values wise the total export of *Litopenaeus vannamei* alone contribute approx. 329766 metric tons (MPEDA, 2017).

Among all the species of shrimp, *Litopenaeus vannamei* represent over 90% of shrimp culture in the western hemisphere and presently it is the most commonly cultured shrimp in Central and South American countries, China, India and Thailand (Frias-Espicueta *et al.*, 2001; McGraw *et al.*, 2002; Saoud *et al.*, 2003). The natural distribution of this species is mainly in brackish water area where annual rainfall and evaporation cycles expose the species to wide seasonal variations in temperature and salinity. The ability of *L. vannamei* to withstand such an extensive range of salinity is utilized by aquaculturist and it is being cultured at salinity ranging from 0 ppt to 35 ppt.

The plethora of problems associated with *P.monodon* such as WSSV outbreak, lack of SPF seed, international market demand etc. has compelled the Indian government to allow the culture of exotic shrimp *L. vannamei* in 2009 after reviewing the results of the pilot

project by Coastal Aquaculture Authority (CAA). At present *L. vannamei* occupied a major part of India and being cultured in freshwater, brackish water and inland saline water. In India, *L. vannamei* culture is going on at very low salinities (~0-2 ppt) in Godavari and Krishna districts of Andhra Pradesh and Thanjavur district of Tamil Nadu and very high salinities (50-60 ppt) in Tamil Nadu and Gujarat (CIBA, 2017).

India has a total brackish water area of 1.24 million ha and only 20% of it is being utilized. After West Bengal, Gujarat has the second largest area of brackish water land. In Gujarat, shrimp farming is gaining momentum day by day. Presently, approximately 15,000 ha are being used for brackish water shrimp culture and it is expected to grow further. In Gujarat, the major shrimp farming areas are Valsad, Navsari, Surat and Bharuch, of which Surat is taking lead in brackish water aquaculture. Bhavnagar, Amreli and Jamnagar of Saurashtra coast recently catch attention and found suitable for shrimp culture.

Global warming has emerged as a serious problem all around the world. It is further predicted that global warming and a consequent increase in water temperature could impact aquaculture significantly. Exceeding temperature beyond optimal range has a direct impact on salinity due to increased evaporation. Salinity change is highly correlated with many other water parameters such as pH, dissolved oxygen, alkalinity, hardness, nitrite TAN etc. These water quality parameters directly affect growth and survival of organism especially in stressful hyperosmotic environment and further prone to bacterial attack such as *Vibrio alginolyticus*, *V. harveyi* etc. Many parts of Gujarat and Tamilnadu faces sever salinity rise which reaches up to 60 ppt during extreme summer.

Probiotics are being applied extensively in shrimp farming to control the outbreak of disease. Several farmers are using probiotics at higher salinity too. But whether probiotics show their beneficial effects at higher salinity (especially >40 ppt) or not is still under the scanner. Therefore, the aim of the present study is to compare the performance of probiotics on water quality, growth performance and survival of *L. vannamei* stocked at higher salinities.

With this background information, present research work was designed to compare the performance of probiotics on water quality, growth performance and survival of *L. vannamei* stocked at higher salinities.

2. MATERIALS AND METHODS

The experiment was conducted for 60 days to compare the growth performance and survival of *Litopenaeus vannamei* at higher salinities with probiotic. The materials used and methodology adopted for present research work is described as following.

Experimental Site

The experiment was conducted at Ratnakar Aquaculture farm, Rajula, District Amreli, Gujarat. Water used for the culture was collected from the creek and brine water was collected from salt pan located at Victor village, Rajula. The laboratory work was carried out in the farm laboratory. The microbiology work was performed at Regional Research Centre, ICAR- Central Institute of Fisheries Technology, Veraval, Gujarat.

Experimental Animals

Litopenaeus vannamei Post-larvae-12 (PL-12) was procured from “West Coast Frozen Food Pvt Ltd.” Shrimp Hatchery Division, Kotda (20° 41' N, 70° 50') (Ta. Kodinar, Dist: Gir-Somnath). PL were acclimatized and stocked in the pond and grown up to 2.5 to 2.9 gm. Shrimps of average size 2.9-3.0 gm. were transferred into the tank with different salinities (according to treatment) for acclimatization for 10 days before the start of research. Only healthy and active shrimps of average size 3-3.1 gm were stocked in the experimental tank with stocking density 45 nos per tank.

Experimental setup

The experiment was carried out in square plastic tank of 1000 litre capacity. The experiment was set up following a completely randomized design. The experiment included one control and three treatment groups in triplicates following Completely Randomized Design (CRD) such as 45 ppt salinity to rear shrimps with probiotic supplementation (control), 50 ppt salinity to rear shrimp with probiotic supplementation (T-1), 55 ppt salinity to rear shrimp with probiotic supplementation (T-2), 60 ppt salinity to rear shrimp with probiotic supplementation (T-3). Experimental tanks were washed with potassium permanganate solution (5 ppm) and sun-dried before the start of the experiment. All the tanks were half filled (around 500 liters) with water of desired salinity by mixing creek water and brine (Helm and Bourne, 2004). Tank water was further disinfected by bleaching powder @ 60 ppm and then supplied with 4 air stone-hoses type of diffuser system connected to 2 HP blowers for vigorous aeration. Aeration was provided throughout the experimental duration except during feeding and sampling. Zero water exchange system (with intermittent water addition of desired salinity to maintain water volume in all tank) were followed during the whole experimental period of 60 days.

Experimental Diet and Feeding

Animals were fed at the rate of 5 % of their body weight during the experiment with commercially available feed (CP) having crude protein 35%, crude fat 5%, fiber 4%, and moisture 11%). Feeding frequency was four times a day similar to actual shrimp farm at 07:00 AM (morning), 11:00 AM, 3:00 PM (afternoon) and 7:00 PM (evening). Feed Probiotic (*Bacillus* spp) was incorporated into the feed along with binder @ 10gm/ kg. The same probiotic added feed was used in all treatments and control.

Physico-chemical Parameters of water

Different water quality parameters such as dissolved oxygen, salinity, pH and temperature were measured at regular intervals. Total hardness, total alkalinity, total ammonia nitrogen (TAN) and nitrite were measured weekly following standard protocol. The concentration of dissolved oxygen was determined weekly using the Winkler titration method and expressed as mg/l. The salinity of water was measured using refractometer (Atago, Ltd, Japan) and expressed in ppt. pH and temperature were measured by using pH meter and thermometer (Hanna, Portugal). Total ammonia concentration was measured weekly by using spectrophotometer at a wave length of 640 nm following Phenate method (APHA, 1998) and compared with the standard graph. The concentration of ammonia was expressed in mg/l. Nitrite level of water was measured as per Phenate method (APHA, 1998) at 543 nm using a spectrophotometer and it was compared with the standard graph of nitrite nitrogen concentration. The concentration of nitrite was expressed in mg/l. Total alkalinity was measured using a standard titration method and it was expressed in mg/l. Total hardness was measured using a standard titration method and it was expressed in mg/l.

Growth Parameters

Randomly 30% of shrimp in every tank were sampled fortnightly for the collection of data required for estimation of growth parameters. The weight of animal was measured by using electronic balance. The utmost care was taken while sampling to minimize stress on the animal. Following growth parameters were estimated.

$$\text{Average body weight} = \frac{\text{Total body weight of shrimp}}{\text{Number of shrimp}} \quad (1)$$

$$\text{Mean weight increment} = \text{Final average body weight} - \text{Initial average body weight}$$

SGR (specific growth rate) as a percentage was calculated using the formula given below. (2)

$$\text{SGR} = \frac{\text{Log}_e(\text{Final weight}) - \text{Log}_e(\text{Initial weight})}{\text{Number of days}} \times 100$$

Food conversion ratio (FCR) is the weight of the food consumed divided by the body weight gain, all over a specified period of time. The FCR (Food Conversion Ratio) was calculated using the following formula: (3)

$$\text{FCR} = \frac{\text{Amount of feed given (g)}}{\text{Body weight gain (Wet weight)(g)}} \quad (4)$$

The feed efficiency ratio was calculated using the following formula (5)

$$\text{FER} = \frac{\text{Body weight gain (wet weight)(g)}}{\text{Feed given (Dry weight)(g)}} \quad (6)$$

3. RESULTS AND DISCUSSION

Sixty days experiment was carried out to compare the growth performance and survival of *L. vannamei* cultured at different higher salinity (45, 50, 55 and 60 ppt) with probiotics. Parameters observed and other results obtained during the experimental period along with discussion are presented following.

Physico-chemical Parameters

Water quality parameters were measured at regular intervals and presented comprehensively in table no. 1 (control and T-1) and table no. 2 (T-2 and T3). Temperature plays a critical role in shrimp physiology and affects final growth performance hence monitored regularly. Temperature observed during the experiment was found in the range of 19.76±0.304°C to 25.46±0.128°C. A decrease in temperature during the experimental period was observed in all tanks. Observations are presented in table 1 & 2 and graphically illustrated in fig. 1. The mean value of temperature in control, T-1, T-2 and T-3 was found to be 22.38±0.165 °C, 21.74±0.281 °C, 21.93±0.276 °C and 21.88±0.341 °C respectively. The range of pH recorded during the experiment was 7.4±0.057 to 8.2±0.057. Values of pH

recorded throughout the experiment and presented in table 2 and 3 and graphically illustrated in fig.2. The mean value of pH in control, T-1, T-2 and T-3 were found to be 7.85 ± 0.044 , 7.77 ± 0.039 , 7.70 ± 0.041 and 7.65 ± 0.041 respectively. Total hardness was estimated weekly and presented in table no. 1 and 2. It is also graphically represented in figure 3. The range of the total hardness during the experiment was 8816.70 ± 60.644 to 14717 ± 948.338 mg/l. The mean value of total hardness in control, T-1, T-2 and T-3 were found to be 9768.18 ± 105.454 , 10642.52 ± 553.564 , 10642.52 ± 553.564 and 13284.11 ± 414.327 respectively. Total alkalinity was estimated weekly during the experiments and presented in table 1 and 2 and illustrated graphically in fig. 4. The range of the total alkalinity during the experiment was $130.7.637\pm 1.666$ to 266.67 ± 19.220 mg/l. The mean value of total alkalinity in control, T-1, T-2 and T-3 were found to be 170.18 ± 2.601 , 193.14 ± 15.910 , 209.44 ± 4.446 and 233.88 ± 11.654 respectively. Total Ammonia Nitrogen was estimated weekly and observations are presented in table no. 1 and 2 and the same is illustrated graphically in fig. 5. The range of the total ammonia nitrogen during the experiment was 0.10 ± 0.000 to 0.40 ± 0.100 mg/l. The mean value of total ammonia nitrogen in control, T-1, T-2 and T-3 were found to be 0.20 ± 0.002 , 0.22 ± 0.002 , 0.23 ± 0.001 and 0.20 ± 0.002 respectively. Nitrite was estimated weekly during the experimental period and observations are presented in table no. 2 and 3 and the same is illustrated graphically in fig. 6. The range of the nitrite during the experiment was 0.05 ± 0.000 to 0.20 ± 0.043 mg/l. The mean value of nitrite in control, T-1, T-2 and T-3 were found to be 0.10 ± 0.003 , 0.11 ± 0.003 , 0.11 ± 0.004 and 0.10 ± 0.004 respectively. A constant dissolve oxygen level of 5.00 ± 0.00 to 6.00 ± 0.00 mg/l is maintained during the experimental period in all the tanks. It was observed routinely and aerations were adjusted accordingly to maintain the optimum level. Constant salinity in each tank according to treatment and control (45, 50, 55 and 60 ppt) was maintained throughout the experimental period. It was precisely monitored, and actions were executed in case of any fluctuations observed.

Table 1 Physico chemical parameters of water observed during the experiment in control and T-1

	DOC	Sampling	Temperature (oC)	pH	Total Alkalinity (ppm)	Total Hardness (ppm)	TAN (ppm)	Nitrite (ppm)
Control	1	S1	25.46±0.128	8.20±0.057	130.00±7.637	9816.70±250.355	0.30±0.000	0.15±0.000
	8	S2	24.61±0.176	8.03±0.033	136.67±1.666	10080.00±117.898	0.23±0.008	0.13±0.004
	15	S3	23.72±0.073	7.90±0.152	151.67±1.666	10627.00±57.831	0.26±0.001	0.13±0.005
	23	S4	22.87±0.058	7.86±0.033	188.33±1.666	12320.00±251.661	0.23±0.007	0.13±0.006
	30	S5	22.16±0.123	7.80±0.152	185.00±2.886	8816.70±60.64468	0.16±0.001	0.07±0.008
	38	S6	21.49±0.187	7.76±0.033	185.00±2.886	9120.00±0.000	0.30±0.000	0.15±0.001
	45	S7	20.98±0.268	7.76±0.033	183.33±1.666	9153.30±33.333	0.16±0.003	0.08±0.004
	52	S8	20.28±0.220	7.76±0.033	188.33±1.666	8890.00±142.244	0.10±0.000	0.05±0.002
	60	S9	19.84±0.249	7.66±0.033	183.33±1.666	9090.00±35.118	0.13±0.002	0.06±0.001
T-1	1	S1	25.10±0.204	8.16±0.066	148.33±15.898	10850.00±281.484	0.30±0.000	0.15±0.000
	8	S2	24.18±0.226	7.86±0.033	155.00±15.000	10880.00±216.564	0.33±0.004	0.15±0.003
	15	S3	23.31±0.251	7.86±0.033	161.67±14.240	11783.00±178.916	0.23±0.003	0.11±0.005
	23	S4	22.60±0.175	7.76±0.033	221.67±39.826	12483.00±319.392	0.23±0.009	0.11±0.006
	30	S5	22.03±0.300	7.70±0.057	211.67±14.240	9856.70±144.952	0.30±0.000	0.15±0.006
	38	S6	21.19±0.388	7.73±0.033	226.67±15.898	10120.00±109.697	0.30±0.002	0.15±0.002
	45	S7	20.64±0.313	7.73±0.033	215.00±10.408	9746.98±318.180	0.13±0.001	0.07±0.003
	52	S8	20.09±0.376	7.63±0.033	183.33±7.264	9880.00±381.095	0.13±0.001	0.05±0.004
	60	S9	19.89±0.293	7.50±0.000	215.00±10.408	10183.00±31.797	0.10±0.000	0.05±0.002

*Values are presented as mean ± SE.

Table 2 Physico chemical parameters of water observed during the experiment in T-2 and T-3

	DOC	Sampling	Temperature (°C)	pH	Total Alkalinity (ppm)	Total Hardness (ppm)	TAN (ppm)	Nitrite (ppm)
T-2	1	S1	24.85±0.190	8.06±0.033	153.33±1.666	11813.00±57.831	0.30±0.000	0.15±0.000
	8	S2	23.80±0.113	7.86±0.033	170.00±5.773	13050.00±1185.430	0.40±0.005	0.20±0.004
	15	S3	23.20±0.254	7.80±0.057	183.33±6.009	12590.00±35.118	0.26±0.001	0.13±0.004
	23	S4	22.54±0.187	7.70±0.057	238.33±4.409	13280.00±88.881	0.23±0.000	0.13±0.006
	30	S5	21.79±0.269	7.73±0.033	226.67±3.333	10883.00±31.797	0.23±0.001	0.11±0.008
	38	S6	21.08±0.390	7.73±0.033	223.33±3.333	10850.00±35.118	0.26±0.001	0.13±0.005
	45	S7	20.20±0.378	7.56±0.033	223.33±3.333	13047.00±466.917	0.13±0.001	0.07±0.002
	52	S8	20.04±0.386	7.50±0.000	243.33±8.819	11080.00±135.031	0.13±0.002	0.08±0.001
	60	S9	19.85±0.319	7.43±0.066	223.33±3.333	11017.00±115.662	0.13±0.001	0.07±0.004
T-3	1	S1	24.30±0.000	8.06±0.033	175.00±2.886	14717.00±948.338	0.30±0.000	0.15±0.000
	8	S2	24.09±0.353	7.80±0.000	193.33±6.666	13117.00±349.301	0.33±0.004	0.16±0.005
	15	S3	23.17±0.303	7.76±0.033	198.33±14.813	13783.00±331.679	0.30±0.000	0.15±0.004
	23	S4	22.42±0.353	7.73±0.033	266.67±19.220	14520.00±624.500	0.26±0.008	0.13±0.007
	30	S5	21.69±0.378	7.63±0.033	248.33±14.240	11917.00±354.511	0.23±0.002	0.11±0.009
	38	S6	20.98±0.477	7.63±0.033	248.33±14.240	11850.00±321.299	0.10±0.000	0.05±0.005
	45	S7	20.47±0.465	7.46±0.233	248.33±14.240	14683.00±68.394	0.13±0.001	0.07±0.002
	52	S8	20.03±0.433	7.40±0.057	263.33±16.914	12750.00±316.596	0.10±0.000	0.05±0.006
	60	S9	19.76±0.304	7.40±0.057	263.33±1.666	12220.00±0.000	0.13±0.002	0.08±0.004

*Values are presented as mean ± SE

Fig. 1 Trend line of temperature variation in control and treatments during the experiment

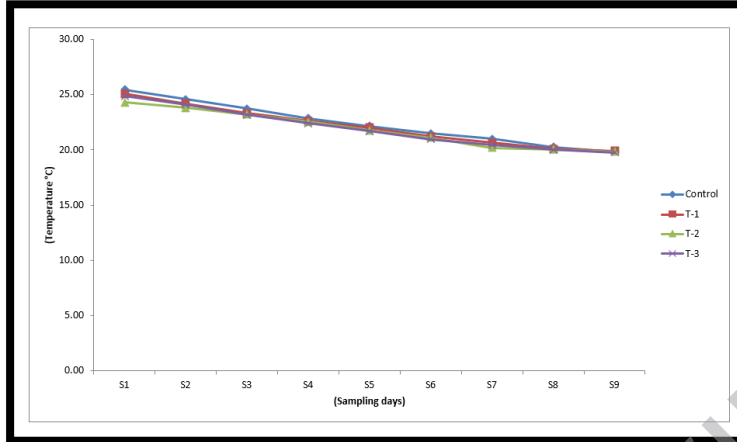


Fig. 2 Trend line of pH variation in control and treatments during the experiment

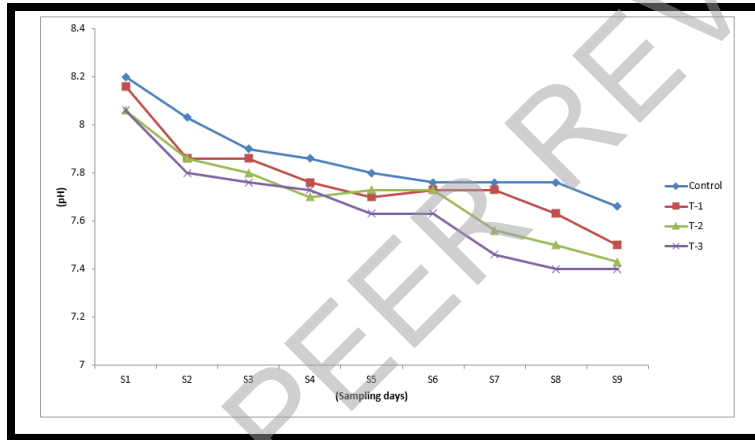


Fig. 3 Trend line of total hardness variation in control and treatments during the experiment

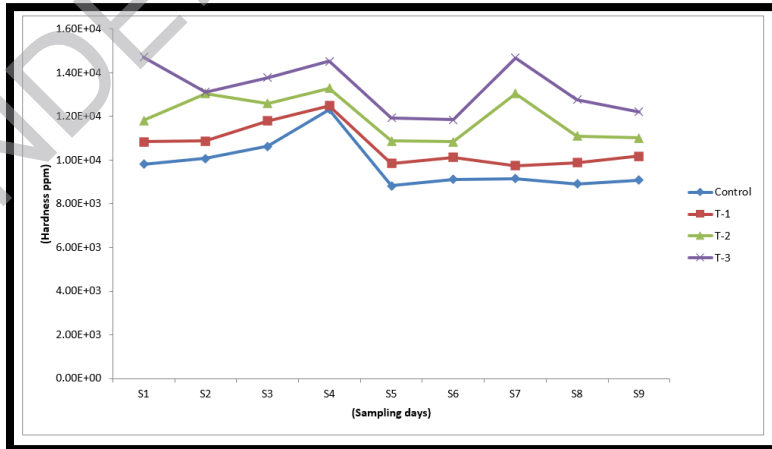


Fig. 4 Trend line of total alkalinity variation in control and treatments during the experiment

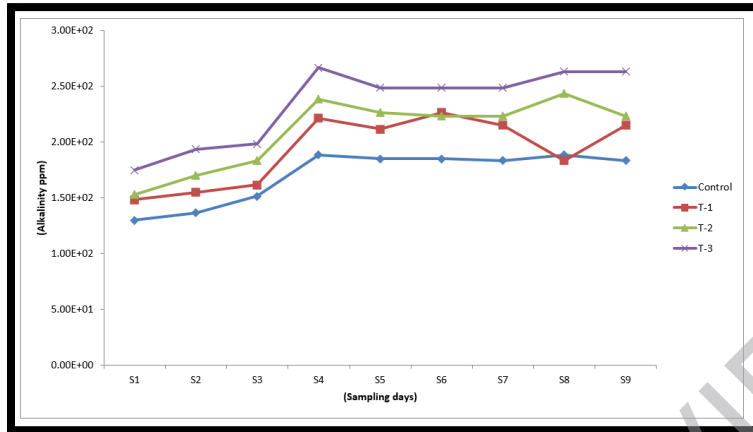


Fig. 5 Trend line of TAN variation in control and treatments during the experiment

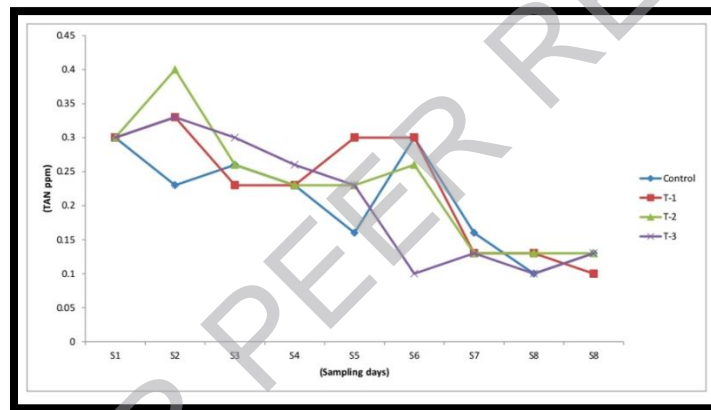
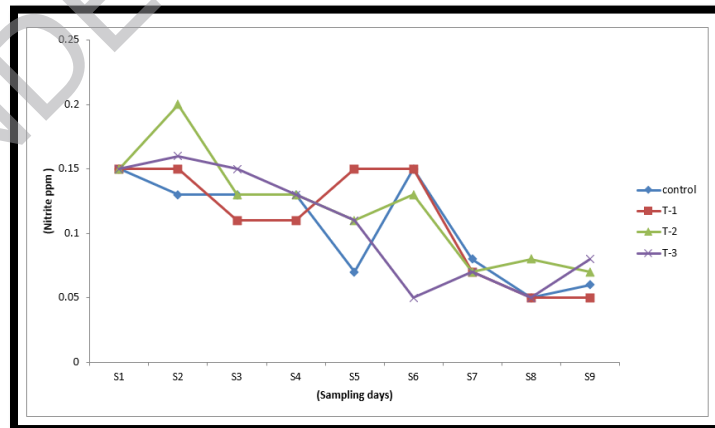


Fig. 6 Trend line of nitrite variation in control and treatments during the experiment



Temperature is one of the most critical parameters in aquaculture systems affecting animal physiology as well as hydrological parameters. Hence it has a direct role on growth performance of the animal. In present experiment, temperature is found to decrease from start (25.46 ± 0.128 °C) to the end (19.76 ± 0.304 °C) due to the onset of winter. The optimal range of temperature for this species is between 30 and 34 °C. At 20°C or lower growth virtually stops (Ravichandran *et al.*, 2012). Similar trends were observed here as the growth performance was poor towards the end of the experiment due to a decrease in temperature. There was no significant difference ($P > 0.05$) in temperature between different treatments and control on different days of sampling except first sampling. pH is also considered as one of the important parameters which affects the physiology as well as soil water chemistry. pH in the range of 7.5-8.5 is considered as the most suitable for aquaculture. Stable day night pH with fluctuation not more than 0.5 is considered the most appropriate for shrimp farming (Ravichandran, 2012; Saraswathy, 2016). In the present study except for few occasions pH was reported in the above said range. Mean pH value in all the treatments and control was found to be almost similar in all the treatments and control (fig. 2). As the decrease in temperature also corresponds to a decrease in pH. Hence in the present experiment, a clear decrease in pH was observed from the beginning towards the end. Hardness is important for shrimp aquaculture as it has a direct effect on moulting. Hardness and salinity are positively correlated. A similar trend is observed in the present experiment (fig. 3) as the mean total hardness was minimum in control and found to increase from T1 to T3. A significant difference ($P < 0.05$) was observed between different treatments and control during most of the sampling day (fig. 3). Alkalinity is an important hydrological parameter in aquaculture and a range of 80-200 mg/l is considered as most suitable for shrimp aquaculture (Ching *et al.*, 2007). Mean total alkalinity observed in the present experiment was found to be 170.18 ± 2.601 , 193.14 ± 15.910 , 209.44 ± 4.446 and 233.88 ± 11.654 for control, T-1, T-2 and T-3 respectively. Hence an increase in total alkalinity is observed along with salinity (fig. 4). A similar finding was reported by (Maica *et al.*, 2014) as they observed an increase in the alkalinity and the concentration of total suspended solids, nitrite and phosphate in *L. vannamei* super-intensive system along with an increase in salinity. A sudden increase in total alkalinity between S3 and S4 was observed due to the addition of water to top up the evaporation loss in each tank. A significant difference ($P < 0.05$) was observed in total alkalinity between different treatments and control on most of the sampling days (fig. 4). TAN concentration is critical for shrimp aquaculture and known to affect it significantly. Most of the efforts in shrimp farm are invested towards minimizing the level of TAN in water. TAN concentrations below 0.1 ppm are recommended in shrimp culture (CIBA, 2017). Here TAN level was found to be in the range of 0.10 ± 0.000 to 0.40 ± 0.100 mg/l. The mean value of total ammonia nitrogen in control, T-1, T-2 and T-3 were found to be 0.20 ± 0.002 , 0.22 ± 0.002 , 0.23 ± 0.001 and 0.20 ± 0.002 respectively. It is reported that the shrimp can tolerate the increased level of total ammonia nitrogen at higher salinity (Kir, M. and Öz, O., 2015). Hence here it can be further concluded that the growth observed even at higher TAN level was due to the same. The lower level of TAN was found in control which may be due to the better action of probiotics applied. Kim *et al.* (2005) reported that *Bacillus* strain has got potential to reduce TAN through nitrification and denitrification. The present study also indicates that TAN level decreased along with culture period which may be due to the better action of probiotic after acclimatization at higher salinity. Nitrite level < 1 mg/l (Ravichandran *et al.*, 2012) is recommended for shrimp farming. Here nitrite level is reported below 1 ppm in all treatments and control. Here the decrease in the level of nitrite was observed from beginning till the end which may be due to better action of probiotic. Dissolved oxygen is critical for aquaculture and level of 4.5 ppm or more is recommended for shrimp aquaculture (Ravichandran *et al.*, 2012). A level of 5.00 to 6.00 mg/l was maintained throughout the experimental period in all the tanks.

Growth Performance

The present study showed better growth performance and yield in control as compared to treatments (T1, T2 and T3). Control showed higher growth performance (ABWG) compared to treatments. Higher PER, FER, SGR, survival and lowest FCR were found in control compared to other treatments. Detailed result related to growth performance was presented in the following section. Average body weight gain (ABWG) of *L.vannamei* reared under control and treatments is presented in table-3 and fig 7. *L.vannamei* showed the highest average body weight gain in control ($3.96\pm0.439\text{gm}$) and minimum in T-3 ($2.57\pm0.001\text{gm}$). Poor growth of shrimp in T3 as compared to control may be attributed due to high saline water, as reported by Zhu *et al.*, (2004) where they observed poor growth performance of *L. vannamei* juveniles at higher salinities. This result is in agreement with the finding of several researchers (Menz and Blake, 1980; Pante, 1990; Bray *et al.*, 1994; Somocha *et al.*, 1998; and McGraw *et al.*, 2002). Survival of *L. vannamei* reared under control and treatment is presented in table-3, figure 8. Survival of *L. vannamei* at the end of the experiment was maximum in control (86.66 ± 0.000) and lowest in the T-3 (63.70 ± 0.000). In the present study maximum survival rate was 86.66% in the control and minimum was 63.70% in T-3. FCR of *L. vannamei* observed in control and treatments are presented in table-3 and fig. 9. Lowest FCR was found in control (1.95 ± 0.327) whereas, maximum in T-3 (12.39 ± 2.806). It may be due to low salinity and the effect of probiotics which provide a conducive environment for the better growth of the organisms. Our finding was supported by the report of improved FCR by use of gut probiotic in the culture of *P.monodon* by Shailender *et al.* (2012). SGR of *L. vannamei* observed in control and treatments are presented in table-3 and fig. 10. A significant difference was observed among different treatments of *L. vannamei*. The highest SGR recorded was in control (1.50 ± 0.114) and the lowest in T-3 (0.9864 ± 0.058). FER of *L. vannamei* observed in control and treatments are presented in table-3 and fig. 11. It was observed that FER is maximum (0.53 ± 0.084) in control whereas minimum in T-3 (0.09 ± 0.024). During the present experiment there was significant different ($P<0.05$) observed in T-2 and T-3 as compared to control. PER of *L. vannamei* observed in control and treatments are presented in table-3 and fig. 12. The value of PER among the treatments and control were significantly different with T2 and T3 respectively. The highest value was observed in the control (0.04 ± 0.004) and minimum in the T-3 (0.02 ± 0.000). In the present study, PER was maximum (0.04) in control and minimum (0.02) in T-3 (60 ppt). The decreasing trends of PER among the treatments compared to control may be due to stressful condition at higher salinities. In this present experiment significant ($P<0.05$) different was observed in T-2 and T-3 as compared to control.

Table 3. Growth performance and survival of *L. vannamei*

	FCR	PER	FER	SGR	ABWG	Survival
Control	1.95±0.327	0.04±0.004	0.53±0.084	1.50±0.114	3.96±0.439	86.66±0.000
T1	2.35±0.684	0.03±0.003	0.51±0.153	1.38±0.113	3.71±0.344	81.48±0.000
T2	6.34±1.798	0.02±0.000	0.18±0.040	1.06±0.092	2.63±0.066	70.37±0.000
T3	12.39±2.806	0.02±0.000	0.09±0.024	0.98±0.058	2.57±0.001	63.70±0.000

Values are presented as mean \pm SE

Fig. 7 Average body weight gain during the culture period

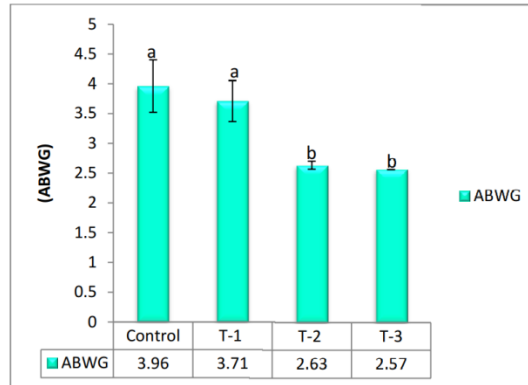


Fig. 8 Survival during the culture period

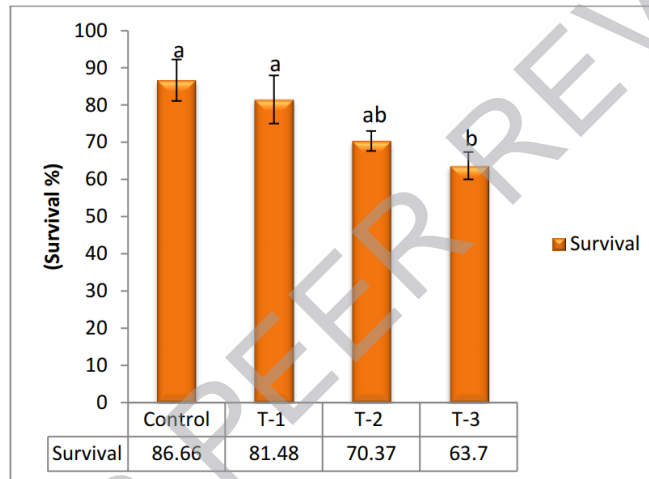


Fig. 9 Food Conversion Ratio during the culture period

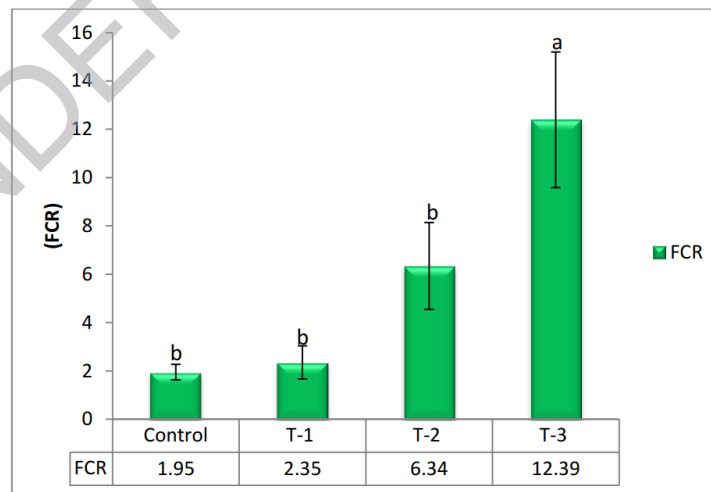


Fig. 10 Specific Growth Rate during the culture period

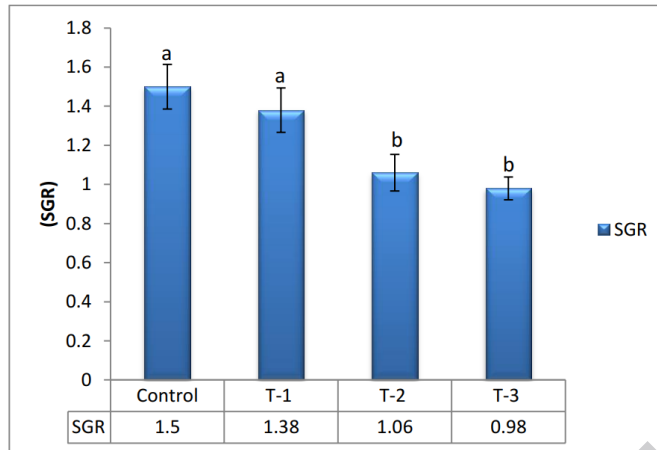


Fig. 11 Feed Efficiency Ratio during the culture period.

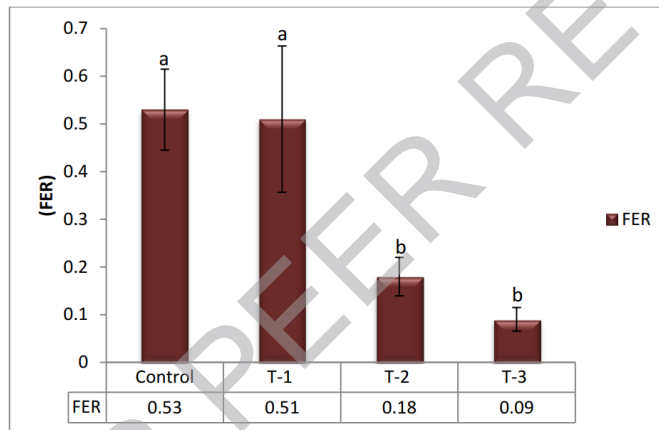
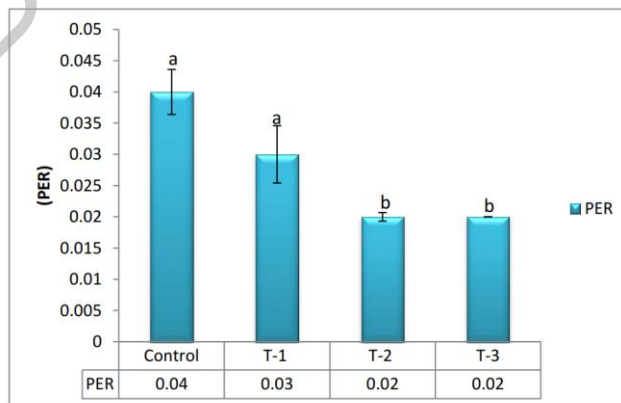


Fig. 12 Protein Efficiency Ratio during the culture period.



According to Bray *et al.* (1994), *Litopenaeus vannamei* achieves maximum growth when reared at salinity levels lower than its isosmotic threshold. The optimal salinity for growth varies among aquatic species and is closely linked to their ability to regulate internal osmotic pressure. Species exhibiting a stronger hyper-osmoregulatory capacity generally perform better under reduced salinity conditions, while those with weaker hyper-osmoregulatory ability tend to show improved growth at elevated salinity levels. Findings from the present investigation revealed that the growth response of *L. vannamei* in Treatment 1 did not differ significantly from that of the control group, indicating comparable performance. The survival report of high mortality in T3 and T4 was in agreement with the finding of Zhu *et al.* (2004) as they demonstrated that a high Na/K ratio in seawater resulted in poor survival of *L. vannamei* and same was supported by the finding of Perez-Velazquez *et al.*, (2007) and Ponce-Palafox *et al.*, (1997) i.e. if salinity increase survival rate is decrease. Here significant different ($P < 0.05$) was observed in T-3 compared with control. FCR may be good due to low salinity and the effect of probiotics which provide a conducive environment for the better growth of the organisms. Our finding was supported by the report of improved FCR by use of gut probiotic in the culture of *P. monodon* by Shailender *et al.* (2012). In present study SGR reported was maximum and minimum in control and in T3 respectively. The obtained results may be due to inclusive effects of probiotic and salinity. Kumar *et al.*, (2013) reported that the use of *B. subtilis* as feed probiotics in shrimps improved growth performance. According to Yan *et al.*, (2007), SGR decreases with increase in salinity. The highest Protein efficiency ratio and feed efficiency ratio were observed decreases with increase in salinity.

4. CONCLUSION

In conclusion, it can be said that probiotic based culture is very well suitable for *L. vannamei* at higher salinity. *L. vannamei* performance was better at 45 to 50 ppt compared to other higher salinities. In addition, probiotic shows better effect at salinity up to 50 ppt as compared to beyond that. Hence farmers can be recommended to maintain a salinity of 50 or lower in the pond for a better harvest. Further research can be performed on the identification of different strains of *Vibrio* Spp. found at higher salinities and also identification of another probiotics bacterium which can show its effect at such higher salinities. Different probiotics combinations can be also identified through research for better performance at higher salinities.

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